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ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

Under the above caption will be published from time to time comments, criticisms and suggestions on technical procedures; minor contirbutions such as laboratory aids and short cuts which are not considered sufficiently important to warrant a formal paper; and queries.

Obviously comments and criticisms should be signed; queries should be signed but names will be withheld on request; full credit will be given those who contribute laboratory aids, short cuts and the like.

An attempt will be made to obtain answers from authoritative sources to the queries submitted. It must be emphasized that the views expressed in this department are not the opinions of any official body.

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GASTRIC ANALYSIS METHODS*

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METHOD OF COLLECTING GASTRIC JUICE

No food or drink is given to the patients between the evening meal and the morning of the test. In the morning, the fasting contents are removed by means of a Rehfuss tube, after which 0.5 mgm. of histamine hydrochloride is injected subcutaneously. *Care must be taken not to get any of the histamine hydrochloride into a vein.* Subsequent samples of gastric juice are collected at 20 minute intervals over a period of 60 to 80 minutes, great care being taken to remove as completely as possible all of the gastric contents at each withdrawal. The patients are advised to take particular care not to swallow saliva.

Each specimen, including the fasting sample, is measured in a graduate cylinder and filtered through a 15 cm. Carl Schleicher and Schüll Faltenfilter No. 1117½. When the volume is very small, centrifugation is used to clear the juice. The presence of blood, bile, and mucus is noted.

The fractional collection of the gastric samples is preferred to the withdrawal of the gastric contents at the end of 1 hour since small amounts of acid which may be secreted during a fraction of the longer period may be neutralized and consequently the value of the gastric analysis as a diagnostic aid will be reduced.

THE DETERMINATION OF pH OF GASTRIC JUICE

The pH of the gastric juice is determined by means of the colorimetric method of Brown, using 1 drop of gastric juice and adding 1 drop of indicator and comparing the resulting color to that produced by a drop of standard buffer solution to which 1 drop of the same indicator has been added.

The determination of the pH is a useful step in the analysis of the gastric contents. The presence of free acid can be determined immediately. In the diagnosis of pernicious anemia this fact alone is worth while. If the pH is above 7.0 the determination of the titratable acid by means of titration with sodium hydroxide can be omitted. Since the enzyme activity in many cases parallels the acid secretion, the determination of pH also aids in selecting the dilutions for the determination of rennin.

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* Prepared at the request of the Editorial Committee.

Solutions. 1. Standard buffer solutions. The solutions are made up at intervals of 0.2 pH from pH 1.0 to pH 8.8, according to Clark.

2. The following indicators are used: Thymol blue pH 1.0–2.8 and pH 8.0–9.6; brom phenol blue pH 3.0–4.6; brom cresol green pH 3.8–5.4; chlorphenol red pH 5.2–6.8; brom thymol blue pH 6.0–7.6; phenol red pH 6.8–8.4.

One-tenth per cent stock solutions of these indicators are prepared as recommended by Clark by grinding 0.1 gram of the dry powder in an agate mortar with the following quantities of 0.05N NaOH and diluting to 100 cc. These stock solutions are stored in glass stoppered pyrex bottles and are diluted as indicated in the following table for determining the pH. For convenience the diluted indicator solutions are kept in dropping bottles.

INDICATOR SOLUTIONS FOR GASTRIC JUICE pH

INDICATOR	TO MAKE 100 CC. OF STOCK SOLUTION		VOLUME OF STOCK SOLUTION DILUTED TO 200 CC. TO MAKE INDICATOR SOLUTION USED
	Indicator	Sodium hydroxide, 0.05 N solution	
	gram	*cc.*	*cc.*
Thymol blue	0.1	4.3	40
Brom phenol blue	0.1	3.0	40
Brom cresol green	0.1	3.2	32
Chlorphenol red	0.1	5.2	20
Brom thymol blue	0.1	3.2	40
Phenol red	0.1	5.7	15

Procedure. A sheet of white glazed paper or filing card is covered with a thin layer of white petrolatum. This allows the drop to stand up and prevents the drop from being absorbed by the paper. Then several separate drops of gastric juice are placed on the vaselined paper and 1 drop of each of the various indicator solutions is added (1 drop of indicator to 1 drop of gastric juice) to determine rapidly in which pH range the gastric juice falls. When the acidity has been narrowed down to the correct indicator range, several drops of the standard buffer mixtures are placed on the paper in close proximity to the drop of unknown gastric juice, and 1 drop of indicator added to each standard and the pH of the gastric juice is estimated by noting which standard buffer it most closely matches.

The pH values found by the authors with the above technique are as follows:

The normal range of pH of histamine stimulated gastric juice at the height of secretion in young healthy adults was from 1.0–1.2.

In patients with true pernicious anemia the pH was always definitely above pH 7.0. Only in rare instances was the pH as low as 7.0 itself.

In idopathic hypochromic anemia the pH varied from 1.0 to 8.3 with a large number of cases having gastric juice with a pH above 7.0.

In anemia due to acute or chronic hemorrhage the pH varied from 1.0 to 4.2 with the majority of cases showing acid values between pH 1.0 and 1.3.

In patients having pellagra with anemia, and patients with other nutritional anemias, the pH varied from 1.0 to 8.6 with the majority of the values between pH 1.1 and 2.3.

In patients with carcinoma of the stomach or with general carcinomatosis the pH varied from 1.0 to 8.0 with a majority of the cases having a pH between 3.0–7.0.

In myxedema the pH varied from 1.0 to 8.2.

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THE DETERMINATION OF TITRATABLE ACID IN GASTRIC JUICE

In the determination of the pH of gastric juice the acidity of many of the samples from pathological cases falls between pH 2.8 and 7.0. If Topfer's reagent or dimethylaminoazobenzene is used as the indicator for the acid titration, these cases would be considered to have an achlorhydria. The end point of pH 7.0 has proved to be more physiological, particularly due to the fact that when the pH is 7.0 or below, pepsin and rennin are present in significant amounts; whereas, when the pH is above 7.0, no pepsin and rennin are present. The following method has been devised in which 1 cc. of filtered gastric juice is titrated to pH 7.0 with 0.01N NaOH, using phenol red as an indicator.

Solutions. 1. Sodium hydroxide 0.1N.

2. Sodium hydroxide 0.01N. This solution is prepared by making a 1:10 dilution of the 0.1N sodium hydroxide. It is standardized daily against 0.1N HCl.

3. Phenol red. A one-tenth per cent water solution of the sodium salt is prepared by dissolving 0.1 gram of phenol red with the aid of 5.7 cc. of 0.05N NaOH and diluting to 100 cc.

4. Phosphate solution pH 7.0. An M/15 phosphate buffer solution of pH 7.0 is prepared by mixing 61.1 cc. of M/15 Na_2HPO_4 with 38.9 cc. of M/15 KH_2PO_4. A few drops of 1 per cent alcoholic solution of thymol are added as a preservative.

Procedure. One cubic centimeter of filtered gastric juice is pipetted into a 60 cc. porcelain evaporating dish and is diluted with about 10 cc. of distilled water. Then 1 to 2 drops of 0.1 per cent phenol red indicator is added and the gastric juice titrated with 0.01N NaOH until the color matches the control solution. The control solution is prepared by adding 5 to 6 drops of the pH 7 phosphate buffer solution to 10 cc. of distilled water in a 60 cc. porcelain evaporating dish and adding the same number of drops of the indicator as was added to the gastric juice.

Precautions. The 0.01N NaOH is standardized on the day used, since at this dilution the normality of this solution may change rapidly.

Calculation. To convert the titration figures into the customary clinical units:

10 A = cc. of 0.1N NaOH required to neutralize 100 cc. of gastric juice.

A = cc. of 0.01N alkali used to neutralize 1 cc. of gastric juice.

THE VALUES FOR TITRATABLE ACID AS DETERMINED BY TITRATION WITH PHENOL RED AS AN INDICATOR

The usual range of titratable acidity in normal young healthy adults was from 80 to 140 cc. of 0.1N HCl per 100 cc. of histamine gastric juice at the height of secretion.

In pernicious anemia there was no titratable acidity.

In idopathic hypochromic anemia the acidity varied from no acid to 121 cc. of N/10. The majority of the cases had no titratable acid and when acid was present the values tended to be low.

In anemia due to acute or to chronic hemorrhage the acidity varied from 6 to 121 cc. of N/10 HCl, with the acid in the great majority of the cases being more than 60 cc. of 0.1N HCl.

In general, in patients having pellagra with anemia, and in patients with other nutritional deficiencies, there was a tend-

ency toward a low titratable acid, although the acid varied from no acid to 144 cc. of 0.1N acid.

In patients with carcinoma of the stomach or with general carcinomatosis the titratable acid varied from 0 to 110 cc. of 0.1N acid. However, there was a definite tendency for low acid values in this group.

In myxedema the titratable acid varied from 0 to 116 cc. of 0.1N acid. There frequently was an absence of titratable acid in this group.

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THE DETERMINATION OF RENNIN IN GASTRIC JUICE

Principle. This method, which is a modification of the one described by Michaelis, depends upon the clotting time of fresh cows' milk to which a solution of calcium chloride has been added. It is rapid and reasonably accurate and has been found to exactly parallel the pepsin concentration in human gastric juice in a large number of analyses. Its simplicity makes it the method of choice for clinical work.

Solutions. 1. Fresh cows' milk, raw or pasteurized.

2. Ten per cent solution of crystalline calcium chloride.

Procedure. The substrate for the rennin is prepared by mixing 90 cc. of cows' milk with 10 cc. of 10 per cent solution of calcium chloride. Two and five-tenths cubic centimeters of this mixture are transferred to a pyrex test tube (15 x 125 mm.) with a lip. By means of a loop of copper wire the tube with the milk is hung over the side of a water bath which is maintained at 40°C. When the tubes have attained the temperature of the bath, gastric juice equivalent to 1/100 cc. to 1 cc., depending upon the rennet activity of the gastric juice, is added to the tubes and the clotting time determined by a stop watch. For gastric juice with a normal acidity usually 0.1 cc. of diluted gastric juice which has been diluted by adding 9 parts of distilled H_2O to 1 part of gastric juice is used. Occasionally with a gastric juice of unusually high enzyme content it is necessary to make a 1:20 dilution instead of the 1:10 dilution. For gastric juice

with lower than normal rennin content, 0.1 cc. to 1.0 cc. of undiluted juice is used.

When adding the gastric juice to the milk it is convenient to hold the tube and the stop watch in the left hand and add the gastric juice from a pipette in the right hand. As quickly as possible after the sample is in the tube, the tube is shaken a few times to mix the gastric juice with the milk and at the time of mixing the stop watch is started. By means of inclining the tube from time to time during the incubation period, the moment of clotting can be readily detected.

The time required to form the clot is converted into "milligrams of rennin" by referring to charts made by plotting the data in the table shown below. These data were obtained by using the above technique with known concentrations of dried U. S. P. rennin. It is best to prepare two charts—one in seconds (0 to 200 seconds) for the higher rennin values and one in minutes for the lower rennin values.

Calculations:

$$\text{Milligrams of rennin per cc. of gastric juice} = \frac{A \times D}{V}$$

A = value in milligrams of rennin found in table or prepared chart for the clotting time in seconds

D = dilution of gastric juice

V = volume of sample of gastric juice added to milk

This method has been standardized against the method for pepsin and it has been found that 10 milligrams of rennin determined by the technique corresponds to 1 mgm. of 1:4000 U. S. P. pepsin when applied to the analysis of human gastric juice.

CLOTTING TIME REQUIRED FOR KNOWN AMOUNTS OF U. S. P. RENNIN

U. S. P. RENNIN	CLOTTING TIME	CLOTTING TIME
mgm.	*seconds*	*minutes*
1.5	9	
1.0	10.5	
0.5	21	
0.3	31	
0.2	44	
0.1	73	
0.075	98	
0.050	145	1.63
0.030	222	3.70
0.020	334	5.90
0.010	650	10.83
0.005	1302	21.70
0.0025		40.00
0.0010		93.00
0.0005		127.00

THE RENNIN CONTENT OF NORMAL AND PATHOLOGICAL HUMAN GASTRIC JUICE BY THE AUTHORS' METHOD

In normal gastric juice 30 to 70 mgm. per cubic centimeter of gastric juice were found.

In pernicious anemia no rennin could be detected in significant amounts unless the juice was contaminated with duodenal contents and then values only as high as 0.1 mgm. per cubic centimeter were found.

Low values were found in carcinoma of the stomach, chronic gastritis and in debilitated states.

Values as high as 130 have been found in patients with ulcer and in other patients in a tense or nervous state.

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THE DETERMINATION OF PEPSIN IN THE GASTRIC JUICE

Pepsin is determined by the method of Koch and McMeekin, a method requiring only 0.5 to 1 cc. of gastric juice, and which depends upon the formation of soluble nitrogen from coagulated and dried egg white. The soluble nitrogen is determined by means of an immersion refractometer or the micro-Kjeldahl nitrogen determination described by Koch and McMeekin. Coagulated egg white is particularly useful in the determination of pepsin in gastric juice, since trypsin from duodenal regurgitation is not able to act upon it to an appreciable extent.

Solutions. Coagulated and dried egg white. Preparation of egg white. In order to eliminate the error due to the absorption of products of peptic hydrolysis by the albumin during coagulation, the egg albumin is coagulated before it is used as a substrate. The coagulated dried egg white is prepared as follows: Fresh eggs are boiled for 15 minutes, allowed to cool, and the shells and yolks

removed. The egg white is washed free from yolk with distilled water and then ground in a meat grinder. The ground egg white is spread over glass plates and dried in a current of warm air. After drying, the coagulated egg white is ground in a mechanical mill and then shaken with an excess of 0.3 per cent HCl for 2 hours at 40°C. The treatment with acid removes inorganic salts, colored

CONVERTING SCALE DIVISIONS OF THE REFRACTOMETER AT 25°C. OR MILLIGRAMS OF SOLUBLE NITROGEN TO MILLIGRAMS OF 1:4000 U. S. P. PEPSIN

PEPSIN	SOLUBLE NITROGEN	CHANGE IN REFRACTIVE INDEX IN SCALE DIVISIONS	PEPSIN	SOLUBLE NITROGEN	CHANGE IN REFRACTIVE INDEX IN SCALE DIVISIONS
mgm.	*mgm.*		*mgm.*	*mgm.*	
0.1	3.30	0.10	4.8	128.6	4.23
0.2	6.64	0.22	5.0	133.5	4.37
0.4	13.76	0.38	5.2	137.5	4.50
0.6	19.60	0.60	5.4	141.8	4.63
0.8	25.60	0.78	5.6	145.9	4.76
1.0	33.20	1.00	5.8	149.8	4.90
1.2	39.50	1.20	6.0	153.0	5.02
1.4	45.60	1.40	6.2	157.5	5.13
1.6	51.80	1.60	6.4	161.2	5.25
1.8	59.60	1.82	6.6	165.0	5.37
2.0	62.90	2.01	6.8	168.5	5.47
2.2	67.80	2.15	7.0	172.6	5.58
2.4	71.20	2.33	7.2	175.8	5.70
2.6	78.20	2.50	7.4	179.0	5.80
2.8	83.40	2.66	7.6	182.7	5.92
3.0	88.60	2.85	7.8	186.4	6.01
3.2	93.10	3.00	8.0	190.6	6.11
3.4	98.00	3.16	8.2	193.0	6.21
3.6	103.20	3.32	8.4	196.4	6.32
3.8	107.30	3.48	8.6	199.6	6.41
4.0	112.40	3.63	8.8	203.2	6.52
4.2	116.50	3.80	9.0	206.4	6.63
4.4	120.80	3.95	9.2	209.4	6.73
4.6	124.70	4.10	9.4	212.5	6.83
			9.6	216.1	6.93
			9.8	219.6	7.05
			10.0	222.9	7.19

extractives and other products which are soluble in 0.3 per cent acid, thereby reducing the soluble nitrogen blank of the egg white. The coagulated egg white is filtered off and washed thoroughly with distilled water. After again drying in a current of air, it is ground so as to pass through an 80 mesh but not through a 100 mesh sieve. About 4 grams of this washed, dried, coagulated egg white are obtained from each egg.

Instead of using eggs, the coagulated egg white can be prepared as follows: Take a 20 per cent solution of Merck's powdered egg white or albumin, heat coagulate it by placing in boiling water for 15 minutes and stir vigorously throughout the coagulation period. The coagulated egg white is then washed with dilute HCl and filtered through cheese cloth, using suction. Further treatment is the same as that for egg white obtained from eggs directly.

Procedure. To determine the amount of peptic activity, 2 grams of egg white are suspended in 49.5 cc. of 0.3 per cent hydrochloric acid in a 125 cc. Erlenmeyer flask, at room temperature. Then 0.5 cc. of gastric juice is added. The solution is shaken and placed in an incubator at 40°C. for two hours where it is rotated vertically during the entire period of incubation. (A water bath and shaker, designed by Doctor Koch, can be obtained from the Arthur H. Thomas Company, Philadelphia.) After the two hour incubation period, the solution is filtered by decantation through 18 cm. quantitative filter paper. The refractive index is determined at 25°C. on the filtrate by means of an immersion refractometer. The difference in refractive index between the unknown and the blank is converted into "milligrams of pepsin" (1:4000) by means of the table prepared by Koch and McMeekin. The blank is set up for each lot of egg white in the same manner as described above for the method. The values in the table are for scale readings of the immersion refractometer. The corresponding values for soluble nitrogen are also shown if an immersion refractometer is not available.

THE PEPSIN CONTENT OF NORMAL AND PATHOLOGICAL HUMAN GASTRIC JUICE BY THE KOCH AND MC MEEKIN METHOD

Normal human gastric juice contained between 3.0 and 6.5 mgm. of 1:4000 U. S. P. pepsin per cubic centimeter.

The gastric juice of patients with pernicious anemia contained no significant amount of pepsin.

Values as high as 13.0 mgm. per cubic centimeter were found in patients having peptic ulcer and in patients in tense and nervous states.

Values as low as 0.3 to 0.5 mgm. per cubic centimeter were found in gastritis and carcinoma of the stomach.

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COMMENTS ON GASTRIC ANALYSIS

LAY MARTIN

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The conventional technique for the estimation of free and so called "combined" hydrochloric acid of the gastric juice by the routine method of titrations to the end point with dimethylamidoazobenzol solution and phenolphthalein has the disadvantage of certain inaccuracies and misconceptions. Recent investigations on the constituents of human gastric juice have shown that its proteins and protein-like material have an isoelectric point of approximately pH 3.5. This fact alone demonstrates the misconception of considering titration from an end point of dimethylamidoazobenzol solution to the alkaline range of phenolphthalein as a measure of combined acidity. The phosphates account in some measure for this titratable range, as also does ammonium chloride. It can be proved that the so called "combined" acidity, which usually measures about 8 to 10 units after a histamine test meal, is accounted for by these three groups of substances. The inaccuracy inherent in the method is in considering titration to an end point of dimethylamidoazobenzol as representative only of hydrochloric acid. Although in great measure this is true, the neutralization of the acid proteins and the phosphoric acid up to pH 4 plays a rôle of some importance, particularly in those instances where the pH of the gastric juice is pH 3 to pH 3.8. It is suggested that in the future the expression "combined" acidity be discarded and that gastric juice be titrated to the end point of dimethylamidoazo-

benzol solution, in this way the hydrochloric acid, "combined" acidity and phosphoric acid of the gastric juice can be determined. For convenience of expression it could be called "the titratable acidity of gastric juice" and it would be understood that this would represent mainly hydrochloric acid. In instances where the titratable acidity was limited to 4 to 8 milliequivalents one might well question the presence of free hydrochloric acid. If it is considered desirable to continue the titration from the end point of dimethylamidoazobenzol solution to the end point of phenolphthalein the result should be considered as representing the buffer strength of gastric juice between pH 4 and pH 8.5. The reagents and solutions and calculations would of course be exactly the same as that used in the conventional titration of gastric juice.

DETERMINATION OF SERUM INORGANIC PHOSPHATE AND OF SERUM PHOSPHATASE*

AARON BODANSKY

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The following is a summary of procedures (described and discussed in detail elsewhere) for the determination of serum inorganic phosphate[1] and of serum phosphatase activity.[2,3] The substrate, sodium beat-glycerophosphate, is hydrolyzed by serum phosphatase to glycerol and sodium phosphate. This substrate is justified particularly, aside from other advantages, by the following consideration: Phosphatase activity is calculated from the difference between (1) total inorganic phosphate, after incubation of the serum with the substrate and (2) serum inorganic phosphate; the latter figure is, however, also of independent importance in most, if not in all, cases where serum phosphatase determinations are required.

STOCK SOLUTIONS

1. *Substrate.* Place successively into a 100 cc. volumetric flask about 3 cc. of petroleum ether (b. p. 20 to 40°C., J. T. Baker Analyzed, Special), about

* Prepared at the request of the Editorial Committee.

80 cc. of distilled water, 0.5 gram of sodium beta-glycerophosphate (Eastman Kodak Research Laboratories), 0.424 gram of sodium diethylbarbiturate (Merck, U. S. P. X), and water to volume (read at interface between substrate and petroleum ether); empty out of doors into a 100 cc. glass-stoppered Pyrex bottle containing about an inch of petroleum ether; keep in the refrigerator. When multiples of 100 cc. are prepared, it is advisable to distribute the substrate into small bottles.

2. *Trichloroacetic acid solution, 10 per cent.* J. T. Baker Analyzed reagent.

3. *Sodium molybdate solution, 7.5 per cent.* Make up in accordance with original directions[1] using "N- and P-free" molybdic acid anhydride, Mallinckrodt reagent quality.

4. *Sulfuric acid, 10 N.* Dilute about 290 cc. of concentrated sulfuric acid (Sp. G. about 1.83, Mallinckrodt reagent quality) to 1 liter; standardize and dilute if necessary; *keep in refrigerator.*

5. *Stannous chloride solution, 60 per cent.* To 15 grams of stannous chloride (J. T. Baker Analyzed) add concentrated hydrochloric acid (Mallinckrodt Analytical Reagent, approximately 36 per cent) to a volume of 25 cc.; *keep in refrigerator.*

6. *Phosphate stock solution.* 250 cc. is to contain 110 mgm. of potassium acid phosphate (LaMotte buffer quality), and 1 cc. of concentrated sulfuric acid; 1 cc. contains 0.1 mgm. of P.

7. *Phosphate standard solution.* Dilute 10 cc. of stock solution to 300 cc.; add a drop of toluene; 6 cc. contain 0.02 mgm. of P.

"Check solutions" of known but arbitrarily varying concentration (one-half to 3 or 4 times that of the standard) may be made by suitable dilution of stock phosphate; preserve with a drop of toluene.

REAGENTS PREPARED DAILY

1. *Acid-molybdate solution.* To the cold 10 N sulfuric acid in a flask add, while mixing, an equal volume of 7.5 per cent sodium molybdate solution; when the mixture is properly prepared it is free of even the slightest tinge of yellow.

2. *Dilute stannous chloride solution.* Dilute 1 cc. of the 60 per cent solution to 400 cc. with water; keep in refrigerator between analyses.

ANALYTICAL PROCEDURES

1. *Collection of serum.* Collect about 5 cc. of whole blood into a centrifuge tube, allow to clot at room temperature, centrifuge, remove serum and centrifuge it. Analysis may be delayed for several hours if the separated serum is kept in ice water or in the refrigerator. If a longer delay is necessary, add a small drop of toluene. After 24 to 48 hours in the refrigerator serum phosphatase activity is about 10 to 15 per cent higher than originally;[2] at room temperature (as in mailed specimens) the results are subject to an error of ±20

per cent.[3] Clinically valid interpretations may, however, be based upon the analyses.

Preparation of filtrates. (1) "Serum inorganic P" filtrate: To 1 cc. of serum in a test tube add 9 cc. of 10 per cent trichloroacetic acid, mix well and filter after a few minutes through 9 cm. filter paper, Whatman No. 44, or other "ashless" grade. (2) "Total inorganic P" filtrate (serum inorganic phosphate *plus* inorganic phosphate liberated from the substrate by the serum phosphatase): Measure 10 cc. of substrate, preferably into a glass-stoppered test tube;* place into a water bath at 37°C. for a few minutes, add 1 cc. of serum, with the tip of the pipette about 1 cm. above the surface of the liquid, invert once or tap to impart rotary motion, mixing the contents well (but without undue aeration); replace into water bath; remove after exactly one hour, cool in ice water for about 2 minutes, add 9 cc. of 10 per cent trichloroacetic acid, mix, let stand a few minutes and filter as above.

When a high phosphatase activity is expected a high dilution is therefore desired, which is accomplished by the addition of larger volumes of 10 per cent trichloroacetic acid. Half-hour or quarter-hour incubation periods may be used when high serum phosphatase activity is expected.

When half quantities are used, about 1 cc. of petroleum ether precedes the addition of 5 cc. of substrate and 0.5 cc. of serum; 4.5 cc. of 10 per cent trichloroacetic acid is added for a dilution of 20, 9.5 cc. for a dilution of 30, etc.

Reagent blanks. Add 2 cc. of acid molybdate solution and 2 cc. of dilute stannous chloride solution, mixing after each addition, to each of two tubes—one containing 6 cc. of water and the other 6 cc. of 10 per cent trichloroacetic acid. The blanks should be colorless or at most tinged faintly green or blue.

Preparation of aliquots for analysis.† The comparison standards (2 or more in each set) contain 0.02 mgm. of P. In accordance with the concentration expected, pipette 2, 4 or 6 cc. of filtrate, and make up with water, if necessary to a total volume of 6 cc. Include in each set some known "check solutions," to verify the accuracy of the determination.

Color development and comparison. To each tube, in sequence, add 2 cc. of acid molybdate solution; mix by tapping, in the same sequence; add 2 cc. of dilute stannous chloride solution to each tube, mixing *during* the addition. The color of the mixture develops rapidly. Colorimetric comparison may be made at convenience—immediately after addition of stannous chloride or at any time within about 2 hours.

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* The glass-stoppered test tubes were made for us by the Scientific Glass Apparatus Co., Bloomfield, N. J. (interchangeable stoppers), and by Eimer and Amend, New York City. Ordinary test tubes and clean rubber stoppers may be employed.

† When colorimeter cups are employed of internal diameter of about 12 mm. or less, aliquots of half the stated volumes may be used with half quantities of the reagents (see table 1).

TABLE 1

INORGANIC P IN ALIQUOTS, AT STATED COLORIMETRIC READINGS, CORRECTED FOR DEVIATION FROM BEER'S LAW, 0.02 MGM. STANDARD SET AT 20 MM.

In column D are given the values for decrements corresponding to an increase of the readings by 0.1 mm., for use in interpolation.

Mm.....	0.0	0.2	0.4	0.6	0.8	D	Mm.....	0.0	0.2	0.4	0.6	0.8	D
mm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		*mm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
5	0.0974	0.0931	0.0892	0.0856	0.0823	18	*21*	0.01890	0.01869	0.01849	0.01829	0.01809	10
6	792	764	737	711	687	12	*22*	1,790	1,771	1,753	1,735	1,717	9
7	665	645	626	607	588	9	*23*	1,700	1,683	1,666	1,650	1,634	8
8	570	554	539	525	512	7	*24*	1,618	1,602	1,587	1,572	1,557	8
9	499	487	475	464	453	6	*25*	1,543	1,529	1,515	1,501	1,487	7
10	443	433	424	415	406	5	*26*	1,474	1,461	1,448	1,435	1,422	7
11	398	390	382	374	367	4	*27*	1,410	1,398	1,386	1,374	1,362	6
12	360	353	346	340	334	3	*28*	1,351	1,340	1,329	1,318	1,307	6
13	328	322	316	311	306	3	*29*	1,297	1,287	1,277	1,267	1,257	5
14	301	296	291	286	282	2	*30*	1,247	1,237	1,228	1,219	1,210	5
15	278	274	270	266	262	2	*31*	1,201	1,192	1,183	1,174	1,166	4
16	258	254	251	248	244	2	*32*	1,158	1,150	1,142	1,134	1,126	4
17	241	238	235	232	229	1+	*33*	1,118	1,110	1,102	1,094	1,087	4
18	226	223	220	217	215	1+	*34*	1,080	1,072	1,065	1,058	1,051	4
19	212	209+	207	204+	202	1	*35*	1,044	1,037	1,030	1,023	1,016	4
20	200	198−	195+	193	191	1	*36*	1,010	1,003	997	991	984	3

Calculations. After colorimetric comparison and calculation in the usual manner from the recorded readings, corrections must be made for the deviation from Beer's law,[1] and for the effects of trichloroacetic acid and glycerophosphate[2,3] (about 1.5 per cent for each cubic centimeter of aliquot used). More rapid as well as more accurate calculation of the results is obtained by the use of tables containing corrected values corresponding to the given readings: The values of the "check solutions," which contain neither trichloroacetic acid nor glycerophosphate, may be obtained from table 1 (milligrams per 100 cc.); the values of "serum inorganic P," in milligrams per 100 cc., are found by consulting table 2, under the division corresponding to the volume of aliquot used; the values of "total inorganic P" are obtained in similar manner, but the dilution in the standard procedure being 20, multiply the "table value" by 2. When dilutions of 30 or 40 are employed, multiply the "table value" by 3 or 4, respectively.

A unit of phosphatase activity is defined as equivalent to the liberation of 1 mgm. of phosphorus (as the phosphate ion) during the first hour of incubation, under the conditions described.

"Total inorganic P" *minus* "serum inorganic P" equals milligrams of "inorganic P liberated" from glycerophosphate by 100 cc. of serum (or units of phosphatase activity per 100 cc. *if hydrolysis continued for 1 hour*).

When serum phosphatase activity is high the relatively large amounts of liberated inorganic phosphate retard the hydrolysis. We have measured this effect, and established a correction, to be applied to the value of "inorganic P liberated," per 100 cc. of serum, during the given period of incubation. A simple rule is: Divide this value by 30, and add the square of the quotient to the uncorrected value. Thus, if 60 mgm. have been liberated per 100 cc. of serum, the correction amounts to 4 mgm., and the corrected value to 64 mgm. per 100 cc.

If half-hour or quarter-hour incubation periods have been employed for hydrolysis, multiply the value of "inorganic P liberated" by 1.82 or 3.3, respectively (more rarely 2 or 3 hour periods may be desirable; in those cases the factors are 0.55 or 0.39, respectively).

In laboratories where phosphatase determinations are made infrequently, the incu' ation period may be kept uniformly at 1 hour, if desired. When the stated correction for retardation of hydrolysis is applied to sera of high phosphatase activity a good approximation to a correct value is obtained. The maximum error would not be great enough to impair the validity of clinical interpretation of the results obtained.

Table 3 indicates, approximately, the range of values of serum phosphatase in the normal state, and in several pathological states in which serum phosphatase is raised. As a technical aid, it may be of some use to include in this table suggestions as to shorter periods of hydrolysis, when these are desired, and the approximate aliquot volumes indicated for the *lower* limit of each range.

TABLE 2

INORGANIC P CONTENT, IN MILLIGRAMS PER 100 CC., CORRECTED FOR DEVIATION FROM BEER'S LAW AND FOR EFFECTS OF TRICHLOROACETIC ACID AND GLYCEROPHOSPHATE

Columns D contain the values for decrements correspondng to an increase of the readings by 0.1 mm., for the use in interpolation.

The values are calculated for a dilution of 10x (as in "inorganic P filtrate"). When the dilution is 20, 30 or 40x (as in "total inorganic P" after incubation), these figures are to be multiplied by 2, 3 or 4 respectively.

When 2 cc. aliquots are used, deduct 3 per cent from the value for the 4 cc. aliquot and multiply by 2.

FOR 6 CC. ALIQUOTS (OR FOR 3 CC. WHEN HALF VOLUMES ARE USED)							FOR 4 CC. ALIQUOTS (OR FOR 2 CC. WHEN HALF VOLUMES ARE USED)						
Mm.....	0.0	0.2	0.4	0.6	0.8	D	Mm.....	0.0	0.2	0.4	0.6	0.8	D
mm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		*mm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
5	17.7	16.9	16.2	15.5	14.9	0.3+	*5*	25.8	24.7	23.7	22.7	21.8	0.5
6	14.4	13.8+	13.4	12.9	12.5	0.2+	*6*	20.9	20.1	19.4	18.8	18.2	0.3
7	12.0+	11.7	11.3	11.0	10.7	0.2−	*7*	17.6	17.1	16.6	16.1	15.6	0.2+
8	10.4	10.1	9.8	9.6−	9.3	0.1+	*8*	15.1	14.7	14.3	13.9	13.5	0.2
9	9.06	8.83	8.61	8.41	8.22	0.10	*9*	13.2	12.9	12.6	12.3	12.0	0.1+
10	8.04	7.87	7.70	7.54	7.38	0.08	*10*	11.7	11.5	11.2	11.0	10.7+	0.1+
11	7.23	7.09	6.95	6.81	6.67	0.07	*11*	10.5+	10.3	10.1	9.9	9.7	0.1
12	6.54	6.42	6.30	6.18	6.07	0.06	*12*	9.52	9.34	9.17	9.00	8.83	0.08+
13	5.96	5.86	5.76	5.66	5.56	0.05	*13*	8.67	8.51	8.36	8.22	8.09	0.07
14	5.47	5.38	5.29	5.21	5.13	0.04	*14*	7.96	7.83	7.71	7.59	7.47	0.06
15	5.05	4.97	4.90	4.83	4.76	0.04	*15*	7.35	7.24	7.13	7.02	6.92	0.05
16	4.69	4.62	4.56	4.50	4.44	0.03	*16*	6.82	6.72	6.63	6.54	6.45	0.05
17	4.38	4.32	4.26	4.20	4.15	0.03	*17*	6.36	6.28	6.20	6.12	6.04	0.04
18	4.10	3.05	4.00	3.95	3.90	0.02+	*18*	5.96	5.89	5.82	5.75	5.68	0.04
19	3.85	3.80	3.76	3.71	3.67	0.02	*19*	5.61	5.54	5.48	5.42	5.36	0.03
20	3.63	3.59	3.55	3.51	3.47	0.02	*20*	5.30	5.24	5.18	5.12	5.07	0.03
21	3.43	3.40	3.36	3.32	3.29	0.02−	*21*	5.02	4.96	4.91	4.86	4.81	0.03−

22	3.25	3.22	3.18	3.15	3.12	0.02−	*22*	4.76	4.71	4.66	4.61	4.56	0.03−
23	3.09	3.06	3.03	3.00	2.97	0.02−	*23*	4.52	4.47	4.42	4.38	4.34	0.02
24	2.94	2.91	2.88	2.86	2.83	0.01+	*24*	4.30	4.26	4.22	4.18	4.14	0.02
25	2.80	2.78−	2.75	2.73−	2.70	0.01+	*25*	4.10	4.06	4.02	3.98	3.94	0.02
26	2.68−	2.65	2.63	2.61−	2.58	0.01+	*26*	3.91	3.87	3.83	3.79	3.76	0.02
27	2.56	2.54	2.52−	2.50−	2.47+	0.01	*27*	3.73	3.69	3.66	3.63	3.60	0.02−
28	2.45	2.43	2.41	2.39	2.37+	0.01	*28*	3.57	3.54	3.51	3.48	3.45	0.01+
29	2.36−	2.34	2.32	2.30	2.28	0.01	*29*	3.43	3.40	3.38	3.35	3.33	0.01
30	2.27−	2.25−	2.23	2.21	2.20−	0.01	*30*	3.30	3.28	3.25	3.22	3.20	0.01
31	2.18	2.17−	2.15	2.13	2.12−	0.01	*31*	3.18	3.15	3.13	3.11	3.09	0.01
32	2.10	2.09	2.07	2.06	2.04		*32*	3.07	3.03	3.02	3.00	2.98	0.01
33	2.03	2.02	2.00	1.99	1.97		*33*	2.96	2.94	2.92	2.90	2.88	0.01
34	1.96	1.95	1.94	1.92	1.91		*34*	2.86	2.84	2.82	2.80	2.78	0.01
35	1.90	1.88	1.87	1.86	1.85		*35*	2.77	2.75	2.73	2.71	2.70	
36	1.84	1.82	1.81	1.80	1.79		*36*	2.68	2.66	2.64	2.63	2.61	

It is beyond the scope of this paper to give further details or discussion of the method or further clinical or experimental data.[4] It is likewise impossible to include a bibliography of data obtained in other laboratories by this method.

We have found normal phosphatase values in fragilitas ossium (without osteomalacia), Ewing's tumor, calcinosis, mongolism, achondroplasia; normal or slightly raised phosphatase values in bone fractures, generalized osteoporosis, osteomyelitis, Gaucher's disease, localized bone cysts, multiple myeloma and giant cell tumors.

TABLE 3

A Summary of Phosphatase Values in Clinical Cases

The figures in parentheses indicate, approximately, the aliquot volumes, in cubic centimeters, suitable at the corresponding phosphatase activities.

	PHOSPHATASE ACTIVITY		DILUTION TIMES	INCUBATION PERIOD
	Minimum	Maximum		
	units per 100 cc.	*units per 100 cc.*		*hour*
Adult, normal	2 (6)	4	20	1
Child, normal	5 (4)	15	20	1
	(6)		20	½
Group 1—Slightly active process	5 (4)	20	20	1
Group 2—Moderately active process	20 (2)	50	20	1
	(4)		20	½
Group 3—Active process	50 (2)	100	40	1
	(4)		40	½
Group 4—Very active process	100 (2)	250	40	½

In our experience, Group 1 included osteomalacia, some cases of hyperparathyroidism, monoostotic Paget's disease of bone (and healed polyostotic disease), "marble bones," osteogenic sarcoma, carcinoma of bone, healing or healed rickets; Group 2 included some cases of monoostotic and polyostotic Paget's disease, hyperparathyroidism, carcinoma of bone, fragilitas ossium with osteomalacia, moderately active rickets; Group 3 included polyostotic Paget's disease, extensivec arcinoma of bone and active rickets; Group 4 included severe polyostotic Paget's disease and severe rickets.

The phosphatase values in clinical liver involvement, with or without jaundice, have frequently been similar to those in Groups 1 and 2.

Anemia and cretinism in children are associated with lower values; adult values are to be expected in sexually precocious children.

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BLOOD GROUPING AND COMPATIBILITY TEST—A SIMPLE AND ACCURATE TECHNIC*

ALFRED S. GIORDANO AND DEANE EAGER McBRIDE

In a recent communication Hoxworth and Ames reported their experience with the combined method of Coca and Vincent as a routine technic in blood typing and compatibility test for the transfusion of blood and pointed out the desirability of a technic which is accurate, simple and rapid. For a detailed discussion of this problem, the reader is referred to their original paper (1).

The purpose of this communication is to point out certain

* From the South Bend Medical Laboratory. Received for publication June 1st, 1937.

dangers in this method based on established immunologic principles and at the same time describe a modified technic which eliminates such dangers and adds to the simplicity of the test. In the technic described by Coca and by Hoxworth, and Ames, no definite statement is made as to the amount of defibrinated blood to be added to the test serum. This is an important consideration since it is well known (2) that the concentration of the cell suspension bears a definite relation to the concentration of the isoagglutinins. In some instances in the presence of an excess number of cells, absorption of specific isoagglutinins occurs without agglutination, particularly in cases with low isoagglutinin titer. This phenomenon actually has occurred during our early experience with this method when we formerly used two drops of serum to one small drop of defibrinated blood. This led to a series of experiments by varying the amount of cells added to the serum to obtain optimum agglutination and we finally came to the conclusion that a loopful (3 mm. in diameter) of defibrinated blood will contain sufficient cells to yield a clean cut agglutination.

We also found it to be more convenient to defibrinate the blood by the use of a small glass bead measuring 3 to 4 mm. in diameter instead of the wooden applicator as described by Hoxworth and Ames, and to use a short test tube measuring 3.5 by 0.9 cm. which is carried about on a tray by placing it in a larger test tube partially filled with cotton. The technic adopted in our laboratory is as follows:

Approximately 10 drops of blood are obtained by deep finger puncture and allowed to run quickly in a small tube preferably 3.5 by 0.9 cm. outside dimensions. Such a tube has been found very convenient for this purpose and is carried to the bedside in a larger tube partially filled with cotton. The blood is defibrinated as it flows into the tube by shaking with a small glass bead measuring about 3 to 4 mm. for about 3 to 5 minutes. When the blood has been shaken sufficiently it will have a lighter foamy appearance. The tube is then placed in a small block of wood in which holes of the proper size have been bored. The bead need not be removed until the test is completed. The blood is well mixed before any is removed for testing.

Grouping. Measure out two or more drops of high titer serums, types A, B, and O, from a roughly calibrated pipet, the amount not to be less than 0.05 cc.

nor exceed 0.1 cc. of serum. Place on a slide and mark accordingly. A platinum loopful (3 mm.) of defibrinated blood is transferred to the type A serum and mixed well with the loop; the loop is then rinsed in water and flamed to remove traces of blood and serum. Another loopful of blood is transferred to the drops of B and O serum in the same manner. Care must be taken to notice in transferring the cells to the serum that if the defibrination is not complete small clumps may be carried over with the loop. These clumps should be noted and excluded from the preparation in order to avoid a false interpretation of results; in our experience this has occurred very rarely and was easily detected. Agglutination is rapid and definite macroscopically, usually occurring within one minute. Slides are examined under low power microscope for weaker reactions. If no agglutination is seen, place the slide under a Petri dish cover together with a piece of wet cotton and observe in 15 minutes under low power microscope for final reading.

Matching. A 50 per cent suspension of recipient's defibrinated blood is prepared in the stem of a white blood cell counting pipet by drawing blood up to the 0.5 mark and then filling to the 1.0 mark with physiological saline solution. This 1:2 dilution with saline is made because agglutination cannot be differentiated microscopically in any greater concentration and because dilution inhibits undesirable rouleaux formation. The diluted blood is deposited on the left end of a glass slide and mixed by means of a jet of air blown from the pipet. An identically prepared suspension of donor's defibrinated blood is placed on the right end of the same slide, and one-fifth of this drop (2 divisions of the white pipet) is transferred to the drop of diluted recipient's blood. (This 1:5 ratio is based on the assumption that 500 cc. is to be transfused to a patient whose blood volume is depleted approximately 50 per cent. It provides a wide margin of safety in transfusions from a "universal" donor since dangerously potent agglutinins will not be completely absorbed in this concentration without manifest agglutination.) The 1:5 mixture of donor's and recipient's blood is then stirred with a platinum loop or glass rod, and the slide is placed under an inverted Petri dish with a piece of wet cotton. After 15 minutes the mixture is agitated and observed microscopically under low power for agglutination. Care must be taken not to confuse rouleaux formation with agglutination. The agglutination will be striking if a grouping error has been made but a little practice is required to estimate the degree of agglutination of recipient's cells by a "universal" donor's agglutinins, in the presence of non-agglutinable cells forming rouleaux. Rouleaux can always be broken up by stirring while agglutination will be intensified. Unless the agglutination is so definite and striking that there is no question of confusion with rouleaux formation, the blood may be pronounced compatible.

SUMMARY AND CONCLUSIONS

1. Blood grouping and cross matching can be done on the same sample of blood.

2. Agglutination is more rapid and definite than when using citrated blood.

3. The method is of particular value in children and patients difficult to bleed by venipuncture.

4. The use of the platinum loop insures uniformity and prevents an excess of red blood cells which might absorb the agglutinins and mask agglutination.

5. The use of a small collecting tube with a glass bead for defibrination is an advantage over the longer tube and wooden applicator in which coagulation may occur as the blood runs in before defibrination can take place.

6. The glass bead is a more convenient means of defibrination than a wooden applicator.

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PUBLISHED PROCEDURES RECOMMENDED FOR TRIAL

A NEW METHOD FOR THE RAPID STAINING OF MYELIN SHEATHS

WILBUR K. SMITH AND BRIAN QUIGLEY, Am. J. Path., **13:** 491. 1937

Procedure. 1. Fix in formalin, alcohol-formalin, or Bouin's fluid (fixations containing copper or chromium salts are not suitable).

2. Imbed in paraffin or celloidin, or frozen sections may be used.

3. Mordant for 15 minutes in 4 per cent aqueous solution of ferric ammonium sulphate ($FeNH_4(SO_4)_2$) prepared from violet crystals.

4. Rinse in 70 per cent alcohol to remove excess of mordant.

5. Place sections in 1 per cent hematoxylin containing 2 to 3 per cent glacial acetic acid by volume. (The 1 per cent hematoxylin solution is made by the addition of distilled water to a 10 per cent stock solution of hematoxylin in absolute alcohol.) The solution containing the sections is kept at or near 55°C. The sections at first stain deeply then differentiate. The depth of staining of the myelin sheaths depends on the time the sections are left in the staining-differentiating fluid. Usually 30 to 60 minutes are required but thin sections may require less time.

6. Place in half saturated solution of lithium carbonate for 5 to 10 minutes.

7. Rinse in tap water.
8. Counterstain or stain for chromophilic substance if desired.
9. Dehydrate in alcohol, clear in xylol and mount in balsam.

Notes. Old formalin fixed material stains satisfactorily.

The ferric ammonium sulphate solution can be used repeatedly as long as it remains clear and without precipitate.

The 10 per cent hematoxylin solution does not require ripening though a ripened solution may be used.

Fifteen minutes in the mordant is ample time, but a prolonged stay (2 weeks) does no harm.

Chromophilic substance may be stained with thionin, toluidin blue, or neutral red.

The authors recommend 1 per cent aqueous solution of neutral red as affording good contrast in addition to staining the chromophilic granules.

In addition to the myelin sheaths the procedure affords a nuclear stain of excellent detail.

The method described can be performed in from ½ to 1 hour, depending on the thickness of the section.

CHEMICAL DEMONSTRATION OF SMALL AMOUNTS OF BLOOD IN THE URINE

KAJ. LARSEN, J. Lab. & Clin. Med., **22:** 935. 1937

Procedure. 1. Determine specific gravity and amount of night urine.

2. The entire portion of night urine is well shaken.

3. If the total night urine amounts to less than 300 cc. only 5 cc. is filtered through ordinary filter paper; if the total night urine exceeds 300 cc., but is less than 500 cc., 10 cc. is filtered; if it exceeds 500 cc., 15 cc. is filtered.

4. When all the sample of urine has passed through, spread the filter paper out on a dry filter paper. When the first filter has lost its shine of moisture, 5 or 6 drops of Gregerson's reagent are placed on center of filter. Gregerson's Reagent: 25 mgm. benzidin and 0.2 gram BaO_2 in 5 cc. of 50 per cent acetic acid.

5. If a blue color appears within one to three seconds, the strength of the reaction is designated by ++ which signifies there are about 50 million erythrocytes in the total night urine. If it takes from four to thirty seconds, the strength is designated by + which indicates from 5 to 50 million.

A blue coloring that appears later than thirty seconds is considered a negative reaction.

If the urine is very cloudy and does not clear on heating or on acidifying with acetic acid, the test is a little less sensative. The test is not made if urine gelattinates on addition of alkali.

If the test is positive on a urine with a specific gravity of less than 1,015, the

patient's fluid intake should be reduced to bring the specific gravity above that figure since the test is more sensative on specimens of low specific gravity, and may give a positive test with normal urines since normal persons may put out a half million erythrocytes in the night urine.

Caution. The Gregerson powder (benzidin + barium dioxide) should not be used after it becomes brown. The color changes more slowly if the powder is kept in black paper capsules.

DETERMINATION OF BLOOD UREA BY DIRECT NESSLERIZATION OF FOLIN-WU FILTRATE

WALTER G. KARR, J. Lab. & Clin. Med., **9**: 329. 1924

Recommended by C. A. PONS

Principle:

Urea is converted into ammonium carbonate by urease, Nesslerized, and compared colorimetrically with a standard similarly treated.

Reagents required:

1. N/12 sulphuric acid
2. Sodium tungstate 10 per cent
3. Nessler's solution
4. Urease paper. To prepare the paper 15 grams of jack bean meal is shaken with 5.0 grams of permutit in 100 cc. of 15 per cent alcohol for 15 minutes using a mechanical shaker, or half an hour by hand. Filter in the ice box over night. Draw pieces of heavy ammonia-free filter paper through the filtrate and hang up to dry. Cut in pieces 1 by 1½ inches.
5. Phosphate buffer solution. Dissolve 14.0 grams of sodium pyrophosphate in N/2 phosphoric acid to make 100 cc. The N/2 phosphoric acid is made by diluting 20 cc. of 85 per cent phosphoric acid to one liter and titrating with 5 cc. of N/10 alkali, using phenol phthalein, to a faint pink. Correct as needed.
6. Stock urea standard: Add 0.1286 grams of urea to 200 cc. of distilled water.
7. Working urea standard: Dilute 5 cc. of stock solution to 100 cc. with distilled water, 5 cc. equals 0.075 mgm. N.

Procedure:

1. Place 5 cc. of neutral Folin-Wu filtrate in a test tube.
2. Add 1 or 2 drops of buffer solution.
3. Add two strips of urease paper.
4. Incubate at 50°C. for fifteen minutes.
5. Treat 5.0 cc. of standard urea solution in a similar manner, transfer with rinsings of water to test tubes graduated at 22.5 and 25 cc. and dilute with water to the first mark.
6. Add Nessler's solution to the 25 cc. mark.

Calculation:

Set unknown on the left at 15 mm. The reading of the standard gives the urea nitrogen per 100 cc. of blood when using 5 cc. of filtrate (0.5 cc. of blood).

ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

WRINKLES IN PARAFFIN SECTIONS. To eliminate wrinkles, float sections in cold water on a slide and dissect away the paraffin surrounding the tissue.

Use low power dissecting microscope to insure margins of tissue against injury.—R. W. CUMLEY, Stain Tech., **11:** 26. 1936.

METHOD OF LOCATING MICROSCOPIC FIELDS. The following method is both simple and efficient.

When a field of particular interest is located, depress the oil immersion lens until it is in contact with the slide (or cover glass). Tilt the microscope, remove the condenser, and ring the lens outline with water-proof ink, or any suitable marking fluid. The objects of interest are thereafter always to be found within the area thus outlined.—FRED. BOERNER.

A SIMPLE MAILING CONTAINER FOR SLIDES. By placing strips of corrugated paper along the sides and bottoms of the 4 by 3 by 1 inch card-board boxes in which slides come from the supply houses, a satisfactory mailing case is obtained at no expense.—H. L. WOLLENWEBER, Arch. Path., **22:** 849. 1937.

TO PREVENT BUMPING. The disagreeable phenomenon of bumping, which so often occurs during the acid digestion of the filtrate in the Folin-Wu U. P. N. determination, can be prevented entirely by placing the tube at an angle of 45 degrees during the first part of the digestion and using quartz pebbles instead of glass beads.—D. R. VENABLE.